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# TECHNICAL MANUSCRIPT 391

# ABSCISSION: THE ROLE OF SENESCENCE

Frederick B. Abeles
Robert E. Holm
Harry E. Gahagan, III

APRIL 1967



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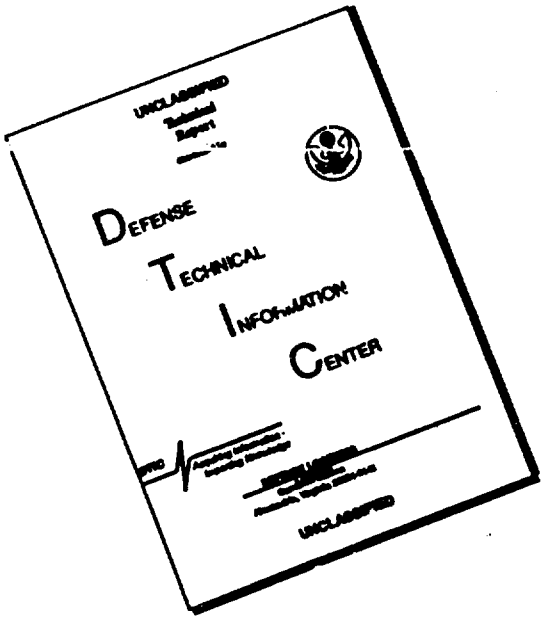


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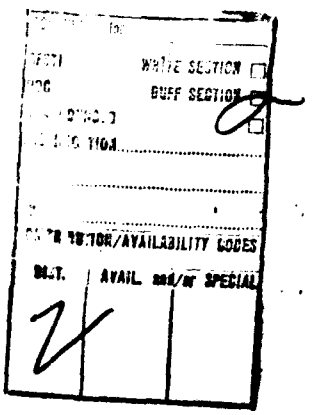

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# ABSCISSION: THE ROLE OF SENESCENCE

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Project 1L013001A91A

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## ABSTRACT

Indoleacetic acid (IAA), coumarin, and the cytokinins 6-furfurylaminopurine, $N^6$-benzyladenine, and 6-benzylamino-9,2-(tetrahydropyranyl-9H-purine) (SD 8339) inhibited the abscission of bean (Phaseolus vulgaris L. var. Red Kidney) petiole explants. IAA, coumarin, and SD 8339 inhibited senescence measured as the loss of chlorophyll, ribonucleic acid (RNA), and protein from the pulvinus. Stimulation of RNA synthesis by ethylene in 22-hour-old explants was inhibited when explant senescence was retarded by IAA or by cytokinin SD 8339. Ethylene stimulated abscission without affecting the degradative processes associated with senescence. Increasing petiole length stimulated the abscission of explants, but addition of sucrose or ethylene masked the effect, suggesting that longer petioles were supplying either more carbohydrates or more ethylene to the abscission zone. Actinomycin D and cycloheximide inhibited degradative changes in the pulvinus, suggesting that catabolic enzymes are newly synthesized after excision of explants.

## I. INTRODUCTION

Earlier papers[1-3] have reported that as abscission zone explants aged they became increasingly sensitive to ethylene, so that at some time after excision the gas hastened cell separation. Aging abscission zone explants are also characterized by mobilization of metabolites from pulvinal tissue into petiole tissue.[4] This paper presents evidence that the action of ethylene appears to be independent of mobilization in the explant. This suggests that senescence encompasses many processes, one of which is the increasing capacity to respond to ethylene while other changes such as mobilization occur in a parallel but independent fashion.

Senescence appears to be a prerequisite to subsequent sensitivity to ethylene, so that any compound that blocks abscission may be acting as a juvenility substance. This idea is supported by reports that auxin[4] and 6-furfurylaminopurine,[5] which are known to block abscission, also retard senescence. Experiments confirming and extending these reports are presented here. Further support is the discovery that coumarin, a compound that blocks abscission, also retards mobilization.

## II. MATERIALS AND METHODS

Seeds of Phaseolus vulgaris L. var. Red Kidney sown in 10-cm pots filled with soil were grown for 14 days at 26 ± 2 C and 1,200 ft-c of fluorescent light (12-hour photoperiod). Abscission zone explants from the primary leaves were incubated in bottles (43 ± 2 ml in volume, 5 cm in diameter, and 2.5 cm in height) fitted with a 25-mm-diameter rubber vaccine cap at 25 C in 400 ft-c of continuous fluorescent light. The 10-mm-long explants (4.5 mm of pulvinal tissue) were placed with the petiole end down in 3 mm of 1.5% agar. When required, ethylene was added to the gas phase with a syringe inserted through the vaccine cap.

### A. DRY WEIGHT

Changes in the dry weight of pulvinal or petiole tissue were determined after the tissue had dried at 98 C for 16 hours.

B. CHLOROPHYLL

Ten petiole or 10 pulvinal sections were homogenized in 10 ml of methanol for 2 minutes at high speed in a VirTis homogenizer fitted with a 50-ml flask. The homogenate was then filtered through glass wool or Miracloth* and centrifuged at 2,000 x g for 10 minutes. The amount of chlorophyll was determined by measuring the optical density of the solution at 666 mμ in a spectrophotometer.

C. RIBONUCLEIC ACID

Ten pulvinal or 10 petiole sections were homogenized in 10 ml of 0.01 M tris buffer [tris(hydroxylmethyl)aminomethane] pH 7.5 with a VirTis homogenizer for 2 minutes and filtered through Miracloth. A 6-ml sample of the filtrate was made 0.2 N with respect to $HClO_4$ and centrifuged at 2,000 x g for 10 minutes. The pellet was washed at 0 to 4 C with 0.2 N $HClO_4$, with 0.05 M formic acid in methanol twice, and at 37 C with ether: ethanol:chloroform (2:2:1 v/v/v) for 30 minutes. The washed pellet was then hydrolyzed in 0.3 N KOH for 18 hours at 37 C. After cooling, sufficient 2.4 N $HClO_4$ was added to give a final concentration of 0.2 N $HClO_4$ and the suspension was centrifuged at 4,000 x g for 10 minutes to yield a clear supernatant. Optical density of the supernatant was measured at 260 and 290 mμ. Milligrams of ribonucleic acid (RNA) were calculated by the relationship: (OD at 260-290 mμ) x (dilution factor) x (0.048).

D. PROTEIN

After samples of tissue were homogenized and filtered as described above to determine RNA, 0.7 ml of 50% trichloroacetic acid was added to a 7-ml portion to coagulate the protein. The protein was precipitated by centrifugation at 2,000 x g for 10 minutes and washed at 0 to 4 C with 5% trichloroacetic acid, with ethanol, and then at 60 C for 5 minutes with ethanol:ether (3:1 v/v). The washed pellet was then solubilized in 1 ml of 0.1 N NaOH. The protein content of the NaOH solution was determined by the method of Lowry et al.[6]

For convenience, the methodology for each experiment is described with the presentation of results because of the variation in procedures among experiments.

* CalBiochem Corp., 3625 Medford St., Los Angeles, California.

## III. RESULTS

### A. MOBILIZATION IN SPLIT AND ETHYLENE-TREATED EXPLANTS

Figure 1 shows the levels of dry weight, chlorophyll, RNA, and protein in petiole and pulvinal tissues 32 hours after the explants were excised, expressed as the percent difference from the initial values. Each point represents the average of four replications, each consisting of 10 explants; the height of the bars represents the mean value of each set of points.

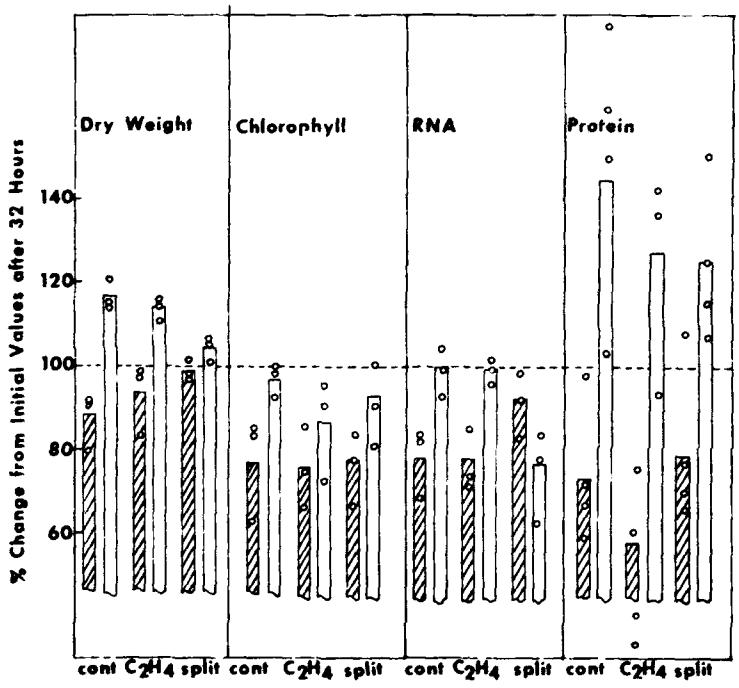


Figure 1. Mobilization of Metabolites in Split and Ethylene-Treated Explants. Each point is the average of four replications of 10 explants each. Height of the bar represents the mean. Content of pulvinal tissue is represented by bars with diagonal lines and petiole tissue with open bars.

The bottles containing abscission zone explants were vented and resealed at 8 and 24 hours to reduce the accumulation of ethylene and $CO_2$. With ethylene-treated explants, 4 nl of ethylene were added per ml gas phase after the explants were excised and after each venting.

The purpose of this experiment was to determine if mobilization and sensitivity toward ethylene were independent processes in senescing explants. If mobilization is a part of the separation process, then any treatment that accelerates abscission should enhance mobilization. After 32 hours, the abscission of ethylene-treated explants was 100% and that of the controls was 30 to 50%. The data in Figure 1 indicate that, although ethylene accelerated abscission, it had no significant effect on the mobilization of chlorophyll, RNA, and protein or on weight changes. Ethylene reduced the protein content of both halves of the explant by 10% although the spread of the data makes the significance of the observation questionable.

Splitting the explants into pulvinal and petiole tissues at the start of the experiment and measuring the changes in metabolite levels after 32 hours should indicate the importance of mobilization in the observed changes. For example, if changes in metabolite levels were the same after 32 hours in intact and split explants, it would indicate that the changes observed were autonomous in pulvinal and petiole tissues and that mobilization (directed transport and accumulation) was not occurring. Our data indicate that this is or was the case with changes in chlorophyll levels.

Changes in dry weight and protein can be partly accounted for by mobilization. Splitting explants reduced the loss of weight from the pulvinus and the gain of weight and protein in the petiole. The changes in RNA levels show mobilization most clearly because dividing the explants in half retarded the loss of RNA from the pulvinus and enhanced the loss from the petiole.

## B. INHIBITION OF ABSCISSION BY INDOLEACETIC ACID, CYTOKININ, AND COUMARIN

Indoleacetic acid (IAA), cytokinin SD 8339 [6-benzylamino-9,2-(tetrahydropyranyl-9H-purine)], and coumarin retarded abscission of bean petiole explants when applied to pulvinal tissue (Fig. 2).

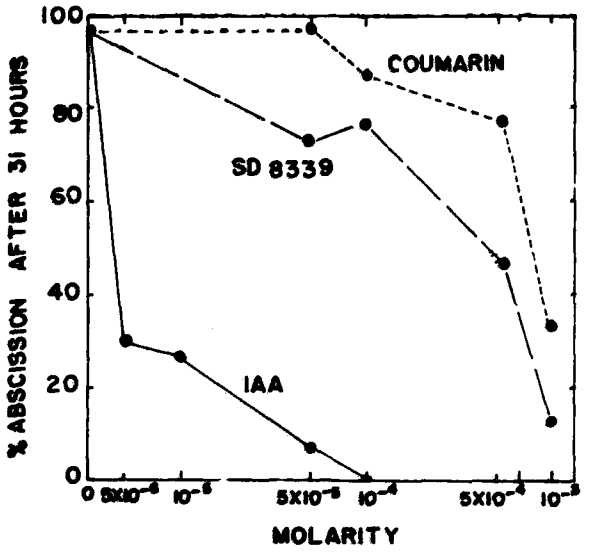


Figure 2. Inhibition of Abscission by IAA, Cytokinin SD 8339, and Coumarin. Each point is the average of three replications of 10 explants each.

Explants were placed pulvinal end down in agar containing various concentrations of these abscission retardants immediately after excision. At 8 and 24 hours the bottles were vented; abscission was measured after 31 hours. Each point in Figure 2 is the average of three replications of 10 explants each. This experiment was repeated on two other occasions with essentially similar results. Two other cytokinins, 6-furfurylaminopurine and $N^6$-benzyladenine, also retarded abscission but were less effective on a molar basis. In addition, at higher concentrations these two cytokinins doubled the rate of ethylene production from explants as contrasted with cytokinin SD 8339, which did not affect ethylene production. We have also found that cytokinins retarded abscission of explants from coleus (Coleus blumei Benth.) and cotton (Gossypium hirsutum L. Acala 4-42) prepared as described earlier.[5] Other growth inhibitors such as juglone, aesculin, naringenin, caffeic acid, ferulic acid, chlorogenic acid, and p-coumaric acid did not inhibit abscission.

## C. EFFECT OF IAA, CYTOKININ SD 8339, AND COUMARIN ON SENESCENCE

Earlier workers have reported that naphthaleneacetic acid[1] and 6-furfurylaminopurine[6] retarded senescence of bean petiole explants. In Figures 3 through 6 these observations have been extended to include the effect of IAA ($5 \times 10^{-4}$ M), cytokinin SD 8339 ($2 \times 10^{-4}$ M), and coumarin ($2 \times 10^{-4}$ M) on levels of dry weight, chlorophyll, RNA, and protein in pulvinal and petiole tissue during abscission.

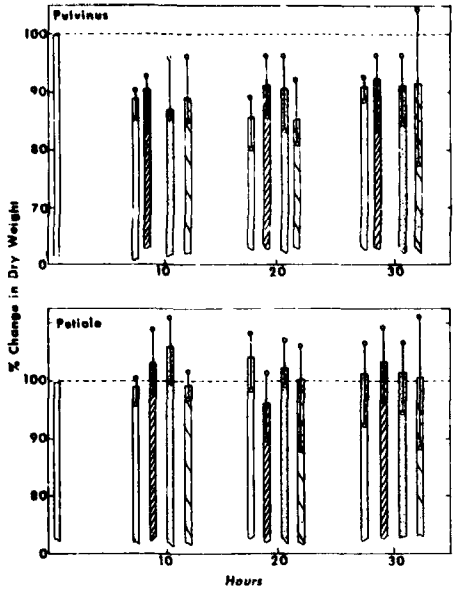


Figure 3. Effect of Abscission Retardants on Dry Weight. Open bar represents control explants. Second bar (diagonal lines) represents explants treated with $5 \times 10^{-5}$ M IAA. Third bar (stippling) represents explants treated with $2 \times 10^{-4}$ M SD 8339. Fourth bar (wide-spaced diagonal lines) represents explants treated with $2 \times 10^{-4}$ M coumarin.

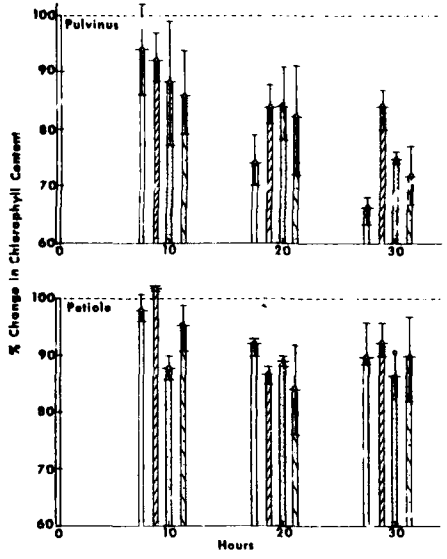


Figure 4. Effect of Abscission Retardants on Chlorophyll Content. For details see Figure 3.

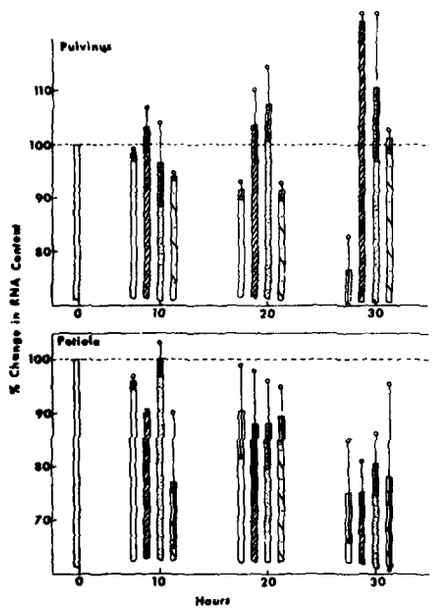


Figure 5. Effect of Abscission Retardants on RNA Content. For details see Figure 3.

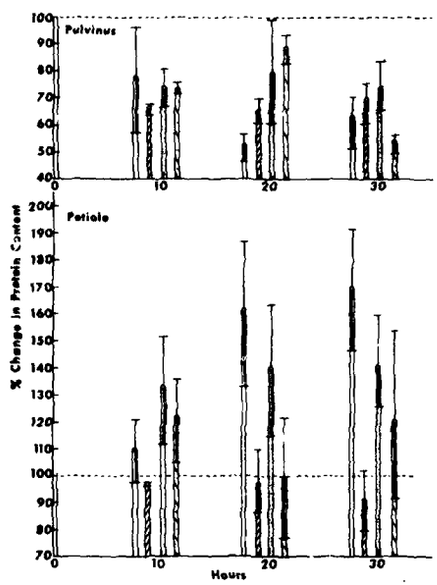


Figure 6. Effect of Abscission Retardants on Protein Content. For details see Figure 3.

TABLE 1. EFFECT OF IAA, CYTOKININ SD 8339, AND ETHYLENE ON $P^{32}$ INCORPORATION INTO RNA

| Treatment | µg RNA, 10 explants | cpm[a], µg RNA | Abscission after 30 Hours, % |
|---|---|---|---|
| Control | 149 ± 3[b] | 2864 ± 7 | 40 |
| Ethylene | 156 ± 5 | 4156 ± 68 | 100 |
| IAA | 173 ± 5 | 4611 ± 102 | 0 |
| IAA + ethylene | 151 ± 6 | 3984 ± 97 | 10 |
| SD 8339 | 200 ± 7 | 4257 ± 81 | 0 |
| SD 8339 + ethylene | 175 ± 4 | 2843 ± 26 | 0 |

a. Counts per minute.
b. Mean ± standard deviation.

## E. PETIOLE LENGTH EXPERIMENTS

Gorter[7] found that increasing the proximal tissue of Coleus rhenaltianus explants accelerated abscission. Scott and Leopold[4] explained that the longer stem might be a more effective mobilization center, thereby accelerating cellular senescence in the abscission zone. However, the acceleration of abscission by larger amounts of proximal tissue may be due to increased availability of carbohydrates or ethylene at the separation zone. Table 2 presents data showing that decreasing petiole tissue inhibited abscission of bean explants but that 1% sucrose or 2 ppm ethylene alleviated the effect. In this experiment, explants with various lengths of petiole tissue were placed petiole end down in either plain agar or agar containing 1% sucrose. The bottles were vented 8 and 24 hours after the start of the experiment and ethylene was added at 24 hours to one series of explants in plain agar.

TABLE 2. EFFECT OF SUCROSE AND ETHYLENE ON ABSCISSION OF EXPLANTS WITH DIFFERENT AMOUNTS OF PETIOLE TISSUE

| | Abscission after 31 Hours, % Petiole Length | | | |
|---|---|---|---|---|
| Treatment | 2 mm | 3 mm | 4 mm | 5 mm |
| Control | 37 | 83 | 87 | 97 |
| Ethylene | 100 | 100 | 97 | 100 |
| Sucrose | 100 | 100 | 100 | 100 |

The abscission retardants were incorporated into agar and the explants were inserted pulvinal end down. Treated explants abscised 0 to 10% after 30 hours; controls usually abscised 30 to 50%. Explants were removed from the bottles after 10, 20, and 30 hours, split into pulvinal and petiole tissues, and frozen. Samples for weight determinations were dried immediately after harvesting. Explants not harvested at 10 and 20 hours were vented at that time.

The data indicate that changes in dry weight were not influenced by the presence of abscission retardants (Fig. 3). Abscission retardants did prevent the loss of chlorophyll and RNA from the pulvinus (Fig. 4 and 5) but they did not affect changes occurring in the petiole. The differences are not as clear as in the earlier examples, but the data in Figure 6 suggest that the abscission retardants prevented the loss of protein from the pulvinus and the increase in protein in the petiole.

## D. INHIBITION OF ETHYLENE-STIMULATED $P^{32}$ INCORPORATION INTO RNA BY IAA AND CYTOKININ SD 8339

We reported earlier[3] that the stimulation of $P^{32}$ incorporation into RNA by ethylene in bean explants depended on an aging process. If aging is required for ethylene action, and if auxin and cytokinins retard aging, then these compounds should also inhibit the effect of ethylene on $P^{32}$ incorporation into RNA.

Explants for these experiments were excised with 10-mm-long petioles to increase the amount of RNA available for subsequent extraction. The explants were placed pulvinal end down in either plain agar or agar containing $5 \times 10^{-5}$ M IAA or $5 \times 10^{-4}$ M SD 8339 for 22 hours. The explants were then placed petiole end down in plain agar, and 1.5 mm by 4 mm agar cylinders containing 2 μc $P^{32}$ were placed on the pulvinal end for 8 hours. Where indicated, 2 nl ethylene per ml gas phase were added during the incubation with $P^{32}$. The RNA from the explants was extracted and purified by methods described earlier.[3] Results are presented in Table 1.

As in the experiments described above, IAA and cytokinin SD 8339 inhibited abscission and RNA breakdown in explant tissue, and ethylene did not significantly alter RNA levels in explants although it stimulated abscission. As reported earlier,[3] ethylene stimulated the incorporation of $P^{32}$ into the RNA of 22-hour-old explants. However, when the ability of ethylene to stimulate abscission was blocked by auxin and cytokinin, its ability to stimulate $P^{32}$ uptake into RNA was also blocked. We do not know why ethylene caused a reduction of RNA levels and of the rate of $P^{32}$ incorporation in auxin- and cytokinin-treated explants.

## F. EFFECT OF ACTINOMYCIN D AND CYCLOHEXIMIDE ON SENESCENCE

Although no direct evidence is available, we assume that the degradative changes occurring in the pulvinus of explants are the result of enzymatic activity. These enzymes are either newly formed or are already synthesized and require only release or addition of a prosthetic group for activation. One way of selecting between these alternatives is to measure the effect of RNA or protein synthesis inhibitors on the decrease of metabolites in explant pulvinal tissue.

If newly synthesized enzymes are characteristic of senescence, blocking RNA or protein synthesis should prevent the degradative changes associated with senescence. Table 3 presents data showing that an inhibitor of RNA synthesis (actinomycin D) and an inhibitor of protein synthesis (cycloheximide) did reduce the loss of dry weight, chlorophyll, RNA, and protein in explant pulvinal tissue.

TABLE 3. INHIBITION OF PULVINAL SENESCENCE BY ACTINOMYCIN D AND CYCLOHEXIMIDE

| | Actinomycin D | | Cycloheximide | |
|---|---|---|---|---|
| Parameter | Control | Inhibitor | Control | Inhibitor |
| | Per Cent of Initial Value after 24 Hours | | | |
| Dry weight | 87 | 93 | 85 | 88 |
| Chlorophyll | 75 | 78 | 62 | 87 |
| RNA | 79 | 84 | 67 | 97 |
| Protein | 90 | 96 | 76 | 105 |
| | Abscission after 24 hours, % | | | |
| Abscission | 81 | 34 | 78 | 13 |

Explants were treated with inhibitors by injecting a 1-µl solution of either 1 µg actinomycin D or 0.25 µg cycloheximide or water into the pulvinus with a microliter syringe after explants were excised from the leaf. Bottles containing explants were vented after 7 hours; after 24 hours the changes in metabolites were measured.

## IV. DISCUSSION

One of the processes during aging or senescence of explants is cell separation at the junction between pulvinal and petiole tissues. Other readily observable processes include the loss of metabolites from pulvinal tissue, which may consist of either mobilization or autonomous degradative processes not influenced by a subtending metabolic sink. The purpose of the experiment summarized in Figure 1 was to determine the role that mobilization played in the loss of metabolites from the pulvinus and to establish the extent to which the cell separation process was autonomous from the loss of metabolites from the pulvinus.

Mobilization and degradation can be distinguished by comparing changes in metabolites in split versus intact explants, because splitting explants into pulvinal and petiole tissues removes the metabolic sink. The results in Figure 1 suggest that the best example of mobilization was the changes in RNA levels. In this case, splitting explants maintained RNA levels in the pulvinus and caused a loss in the petiole compared with intact controls.

Chlorophyll changes were not significantly affected by splitting, indicating that decreased chlorophyll levels are not caused by mobilization. Intermediate in effect were changes in dry weight and protein content. Examining these parameters shows that splitting explants reduced but did not prevent the loss in pulvinal tissue and the gain in petiole tissue, an indication that only part of the observed changes can be attributed to mobilization.

Scott and Leopold[4] carried out similar mobilization experiments for 96 hours; those reported here were terminated after 32 hours. Our controls had started to abscise by that time, so experiments of longer duration would not have measured changes directly related to abscission or at least that part associated with separation, but would have been concerned with secondary phenomena such as cell division in the petiole tissue. Gawadi and Avery[8] suggested that, although cell division and the resultant formation of a protective leaf scar is a part of the overall abscission process, it is not a necessary part of the cell separation process. In support of their findings we have found that inhibitors of DNA synthesis such as 5-fluorodeoxyuracil, amethopterin, and mitomycin C had no effect on cell separation. In addition, ethylene did not stimulate the incorporation of thymidine-$C^{14}$ into DNA.*

Although ethylene stimulated abscission, our data show that the gas did not stimulate mobilization of any metabolite or result in a decrease in dry weight, chlorophyll, or RNA. Ethylene actually reduced mobilization when expressed as dry weight changes. The ethylene-enhanced explant

* Unpublished results.

separation was in effect similar to splitting explants mechanically but, because it occurred later, the effect was intermediate between results seen in controls and split explants. However, ethylene treatment resulted in a loss of protein in both petiole and pulvinal tissues. There is no obvious explanation for this effect. Our results suggest that the cell separation process and the loss of metabolites from the pulvinus are independent processes characteristic of aging explant tissue.

The stimulation of abscission of coleus explants by long petioles reported by Gorter[7] was cited by Scott and Leopold[4] as evidence in favor of the idea that a more effective (larger) mobilization center would hasten senescence and thereby abscission. However, the results shown in Table 2 suggest that ethylene production or availability of carbohydrates may be other explanations for the effect of explant size on abscission.

IAA, cytokinins, and coumarin retarded abscission. Because these three compounds are normally thought to control different aspects of plant metabolism, i.e. growth, cell division, and growth inhibition, respectively, we explored the reasons for their ability in common to block abscission.

Although the inhibition of abscission by coumarin has not been described earlier, the result is not completely unexpected, as there are a number of examples where auxin and coumarin have similar effects on plants. Audus and Quastel[9] found that both 2,4-D and coumarin inhibited the growth of cress and carrot seedlings, but that coumarin inhibited seed germination and 2,4-D did not. Winter[10] reported that both auxin and coumarin stimulated the lateral growth of decapitated sunflower seedlings. More recently, Gantzer[11] found that both coumarin and IAA stimulated the elongation of oat coleoptile sections at low concentrations and inhibited it at higher concentrations. However, coumarin did not stimulate growth in the oat curvature test nor did it appear to be transported in a polar manner.

The effect of coumarin may be auxin-sparing, like that described for phenolic compounds by Tomaszewska[12] and Schwertner and Morgan.[13] Those workers found that certain phenols enhanced, but others inhibited, IAA decarboxylase and abscission.

Both naphthaleneacetic acid[4] and 6-furfurylaminopurine[8] have been shown to retard senescence in bean explants. The experiments presented in Figures 3 through 6 confirm and extend these earlier observations with different but closely related compounds.

The retardants had no effect on the weight loss of pulvinal tissue, suggesting that some of the loss represents reserve carbohydrates or other metabolites used to supply energy to the explant during senescence. As

opposed to the trends shown in Figure 1, the petiole tissue did not increase in weight with time. This difference may be because the explants used for the experiments summarized in Figure 1 were placed petiole end down in the agar and those used in the abscission retardant experiments were placed pulvinal end down.

However, the data in Figures 4, 5, and 6 indicated that the retardants did inhibit the breakdown and subsequent loss of chlorophyll, RNA, and protein from the pulvinus. IAA not only prevented the loss of RNA from the pulvinus but caused an increase compared with initial levels.

The retardants had little or no effect on the changes in RNA and chlorophyll content of petiole tissue. Even though retardant-treated explants failed to abscise by 30 hours, the levels of chlorophyll and protein in the petiole were similar to those in explants that were rapidly abscising. This supports the view that some of the changes of metabolite levels are autonomous events characteristic of aging excised tissue.

The increased protein content normally associated with aging explant petiole tissue was inhibited by the retardants. This inhibition of protein synthesis was most readily observed with explants treated with IAA. However, Figure 1 indicates that ethylene also decreased protein levels. The possibility remains that part of this auxin effect may be an ethylene effect because auxin has been shown to stimulate ethylene production.[14]

Senescence of the pulvinus is characterized by a loss of dry weight, chlorophyll, RNA, and protein. These are gross changes and as such reflect the total of a large number of reactions consisting in part of the increase in certain components. For example, even though the pulvinus loses protein and RNA, it is still capable of synthesizing new RNA and protein during abscission,[2] measured as the incorporation of $P^{32}$ into RNA and leucine-$C^{14}$ into protein.

To the extent that loss of metabolites is associated with senescence and that abscission retardants inhibited this loss, we can say that the mode of action of auxin, cytokinins, and coumarin in preventing abscission is associated with their ability to act as juvenility substances. Additional support for this idea was furnished by the experiment summarized in Table 1. In this experiment ethylene stimulated $P^{32}$ incorporation into RNA and abscission of 22-hour-old explants. However, when explants were aged in the presence of auxin and cytokinin for the same time, ethylene had little or no effect on abscission and inhibited $P^{32}$ incorporation into RNA. We conclude from this experiment that auxin and cytokinin prevented senescence, thereby preventing ethylene from stimulating $P^{32}$ incorporation into RNA. The rapid rate of $P^{32}$ incorporation into RNA and higher RNA content also indicate the relative juvenility of auxin- and cytokinin-treated explants.

As shown in Table 3, inhibition of RNA and protein syntheses retarded the breakdown of metabolites in the pulvinus, suggesting that the loss of metabolites may be the result of newly synthesized catabolic enzymes rather than the activation or release of preformed proteins. In support of this idea, other workers have reported an increase in degradative enzymes in aging leaf tissue. Sahai Srivastava and Ware[15] reported increased deoxyribonuclease and ribonuclease activities of detached barley (Hordeum vulgare L. var. Wolf) leaves with age. Treatment of leaves with 6-furfurylaminopurine retarded the decline in chlorophyll, RNA, and deoxyribonucleic acid and suppressed the activities of deoxyribonuclease and ribonuclease. Kessler and Engelberg[16] reported that the total RNA content of apple leaves increased during the first 3 weeks of growth, then decreased during the next 9 weeks. Soluble ribonuclease activity, considered to be associated with catabolic activity, increased during the first 8 weeks of growth, then leveled off in activity.

## V. SUMMARY

In conclusion, the evidence presented here indicates that auxin and cytokinins repress the synthesis of catabolic enzymes and the sensitivity to ethylene in bean petiole abscission zone explants. Excision of the explant probably sets the aging process into motion because the normal supply of juvenility factors is cut off when the leaf blade is removed.

In the preparation of explants, metabolites are lost from the pulvinus under the influence of the proximity of a metabolic sink. Although mobilization on such a small scale is interesting and deserves further study, it apparently has no direct relation to the cell separation process, which occurs simultaneously but independently.

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13. ABSTRACT

Indoleacetic acid (IAA), coumarin, and the cytokinins 6-furfurylaminopurine, $N^6$-benzyladenine, and 6-benzylamino-9,2-(tetrahydropyranyl-9H-purine) (SD 8339) inhibited the abscission of bean (*Phaseolus vulgaris* L. var. Red Kidney) petiole explants. IAA, coumarin, and SD 8339 inhibited senescence measured as the loss of chlorophyll, ribonucleic acid (RNA), and protein from the pulvinus. Stimulation of RNA synthesis by ethylene in 22-hour-old explants was inhibited when explant senescence was retarded by IAA or by cytokinin SD 8339. Ethylene stimulated abscission without affecting the degradative processes associated with senescence. Increasing petiole length stimulated the abscission of explants, but addition of sucrose or ethylene masked the effect, suggesting that longer petioles were supplying either more carbohydrates or more ethylene to the abscission zone. Actinomycin D and cycloheximide inhibited degradative changes in the pulvinus, suggesting that catabolic enzymes are newly synthesized after excision of explants.

14. Key Words

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|---|---|
| *Abscission | RNA |
| *Aging | Proteins |
| Beans | Petiole |
| Weight | Pulvinate |
| Chlorophyll | |

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